Characteristics of Thyroxine 5′-Deiodination in Cultured Human Placental Cells

Regulation by Iodothyronines

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Abstract

Human and rat placental homogenates convert L-thyroxine (T_4) to 3,5,3′-L-triiodothyronine (T_3) via a pathway termed type II iodothyronine deiodination. To study regulation of this pathway, cell dispersions were prepared from human placental chorionic-decidual membrane. Dispersed cells deiodinated T_4 and 3,3′,5′-triiodothyronine (rT_3), but not T_3, at the 5′ position. The reaction was only slightly inhibited by 1 mM 6-*n*-propylthiouracil, enhanced by dithiothreitol, and substantially inhibited by 50 nM iopanoic acid. Incubation of the cells in thyroid hormone-depleted medium induced a near doubling of T_4 5′-deiodination in 36–48 h, with a significant rise seen as early as 12 h. Addition of T_4, rT_3, or T_3 to hormone-depleted medium impaired the rise in type II deiodination in a dose-dependent fashion. T_4 and rT_3 were equipotent in this regard, and T_3 was 2–3 times less potent. T_4 was effective in physiological concentrations, 6.5–13 nM in medium containing 10% calf serum, and the effect of T_4 was not due to its conversion to either T_3 or rT_3. In cells with deiodinase activity raised by 48 h incubation in thyroid hormone-depleted medium, addition of T_4, T_3, or rT_3 reversed the increase in 8–24 h. Secretion of prolactin and βhCG by the dispersed cells was not substantially affected by thyroid hormone deprivation. The increase in type II deiodination during thyroid hormone deprivation appears to depend on a signal from the thyroxine molecule, per se, and could potentially defend intracellular, and/or circulating, T_3 pools in pathological states of mild-to-moderate hypothyroxinemia.

Introduction

Human and rat placenta have an iodothyronine 5′-deiodinating pathway that is thiol dependent, largely localized to particulate subcellular fractions, and insensitive to inhibition by 6-*n*-propylthiouracil (PTU)[1] (1, 2). It has an apparent Michaelis constant (K_m) for L-thyroxine (T_4) of 2–3 nM, and T_4 is a better substrate than 3,3′,5′-L-triiodothyronine (rT_3), as judged by a higher ratio of apparent maximum reaction velocity (V_{max}) to K_m for T_4. These reaction characteristics correspond to those of a deiodinating pathway identified in rat pituitary, central nervous system, and brown adipose tissue (3–7), but not, so far, in other tissues. The potential physiological significance of this pathway, termed type II iodothyronine deiodination, and two other deiodinating pathways in rat tissues has been reviewed (8). It is yet not known how many separate enzyme molecules are represented by these pathways, and the relationship of the type II pathway to the recently described low K_m pathway in rat renal microsomes (9) also remains uncertain.

The increase in type II deiodination induced by hypothyroidism in several rat tissues tested in vitro (3, 5, 6, 8) suggests an adaptive function for this enzyme, whereby intracellular 3,5,3′-L-triiodothyronine (T_3) concentrations might be defended in mild-to-moderate hypothyroidism, to a greater extent than predicted from serum T_4 and T_3 concentrations. There is evidence for the operation of this mechanism in the rat in vivo (10–13). Thus far, there is no direct evidence for such an adaptive role for type II deiodination in man. The goals of the present studies were to develop a system to study regulation of iodothyronine deiodination in intact dispersed human placental cells, to verify that intact human placental cells can convert T_4 to T_3 via the same reaction pathway observed in microsomal preparations, and to ascertain whether the T_4 5′-deiodination rate in human placental cells increases in response to thyroid hormone deficiency. We used the outer, chorionic fetal membrane, which has an adherent layer of maternal decidual cells, as the source of cells, because that membrane has the highest T_4 5′-deiodinase activity of the several placental structures tested (1).

Methods

Cell dispersion. Normal human placentas were obtained within 1 h of vaginal or caesarian delivery of healthy, full-term (38–42 wk) infants (preliminary studies have shown that there was no loss in cell viability up to 3 h after delivery). Placentas used for primary cultures were from Caesarian deliveries and were kept sterile.

The amnion was peeled away from the chorionic membrane, to which a layer of decidua adheres, and the latter was washed (6–10 times) with Hanks' balanced salt solution (HBSS) (Gibco Laboratories, Grand Island, NY) until the supernate did not get any clearer, indicating that blood removal was maximized. The chorion-decidual membrane was then minced and submitted to digestion in HBSS with 2 mM $CaCl_2$ and 1 mM $MgSO_4$, using a proteolytic enzyme solution with trypsin 1:250 (Difco Laboratories, Inc., Detroit, MI), DNAse I (Sigma Chemical Co., St. Louis, MO), and collagenase. Collagenases were obtained from Sigma

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1. *Abbreviations used in this paper:* βhCG, beta subunit of human chorionic gonadotropin; CV, coefficient of variation; DTT, dithiothreitol; HSD buffer, 10 mM Hepes, pH 7.0, 0.32 M sucrose, and 10 mM DTT; M-199, medium 199 with Earle's salts; PRL, prolactin; PTU, 6-*n*-propylthiouracil; rT_3, 3,3′,5′-L-triiodothyronine; T_3, 3,5,3′-L-triiodothyronine; T_4, L-thyroxine; TAA, *t*-amyl alcohol/hexane/2 N NH_4OH/5:1:6; THDCS, thyroid hormone-depleted calf serum.

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Chemical Co.) (types IA, II, IV, V, and VII) or from Worthington Biochemical Corp. (Freehold, NJ) (types I and II). Incubations were carried out in a continuous shaking water bath heated to 37°C. The digestion was stopped by addition of medium 199 with Earle's Salts (M-199, Gibco Laboratories) with 10% calf serum (Flow Laboratories, McLean, VA). Cells liberated between 30 and 90 min were passed through a 149-μm nylon mesh and collected. Cell viability, assessed by trypan blue exclusion, was 82–94%. The cellular protein content was 250±50 μg/million cells.

Cell culture. Cell incubations longer than 8 h were carried out under sterile conditions. The cells were maintained in M-199 with 10% calf serum, 1.0% penicillin-streptomycin (Gibco Laboratories), 20 mM Hepes, and 27 mM $NaHCO_3$, pH 7.4, unless otherwise indicated, under an atmosphere of 95% air/5% CO_2 at 37°C. In experiments with up to 2.0 × 10^6 cells/well, a multiwell dish (Costar, Cambridge, MA) with 5-cm^2 wells were used. When more cells were used, they were plated in a 25-cm^2 plastic petri dish (Nunclon-Delta, Roskilde, Denmark). Medium was changed every other day, using 1 ml of medium per 10^6 cells.

5'-Deiodination measurements. Unlabeled iodothyronines (all levorotatory) were obtained from Sigma Chemical Co., and from Henning GmBH (Berlin, Federal Republic of Germany). High specificity [^{125}I]T_4, [^{131}I]T_3, and [^{125}I]3,3',5'-L-triiodothyronine (rT_3), all labeled in the outer ring, were produced in our laboratory (14). The purity of the tracers was >85% and the specific activity of [^{125}I]T_4 and [^{125}I]rT_3 was 3.0 mCi/μg, and that of [^{131}I]T_3 was 3.5 mCi/μg. Three different assays were used to measure 5'-deiodination rates. All samples from particular experiments were assayed together.

Assay A (short-term incubation of intact dispersed cells). Four million cells were incubated for 3 h in 2 ml serum-free medium with 32–320 pM [^{125}I]T_4, 8–80 pM [^{131}I]T_3, and 10 mM dithiothreitol (DTT, Calbiochem-Behring Corp., San Diego, CA), unless otherwise indicated. After the incubations, the media were harvested and the cells collected and extracted with 95% ethanol (>85% efficiency). [^{125}I]T_3 and [^{131}I]T_3 were isolated, from media and cell extracts by descending paper chromatography in *t*-amyl alcohol/hexane/2 N NH_4OH/5:1:6 (TAA) (15) or by affinity chromatography on anti-T_3 antibody-Sepharose (16), followed by descending paper chromatography in TAA. Affinity chromatography was used (usually for medium samples) when counts in the [^{125}I]T_3 spot were 3.5% or less of the total counts in strips spotted directly; at higher percentages, the results were in excellent agreement with direct paper chromatography, whereas below 3.5%, direct chromatography overestimated the [^{125}I]T_3, especially in the background wells. The [^{131}I]T_3 served as recovery standard in the affinity chromatography. Affinity chromatography resulted in a selective enrichment of T_3 of >20-fold, thereby allowing accurate and reproducible quantitation of T_3 blank values down to <0.5%. The total [^{125}I]T_3 in the system was the sum of the [^{125}I]T_3 in the medium plus that in the cell extract. The results of incubations in cell-free wells were used to determine background deiodination (always <1% conversion of the [^{125}I]T_4 to [^{125}I]T_3 with a coefficient of variation (CV) of 2–18%) and the background values were subtracted from the results obtained in the wells containing cells. T_3 degradation during the incubation was assessed by paper chromatography of the ^{131}I-labeled compounds. Incubations were carried out in triplicate wells, and the results were expressed as percentage of [^{125}I]T_4 converted to [^{125}I]T_3. Because only one of the two outer ring iodines of [^{125}I]T_4 was radiolabeled, the observed percentage conversion of T_4 to T_3 was multiplied by two, to correct for the equal amount of unlabeled T_3 produced. The lower limit of sensitivity for this assay was 0.5% conversion/10^6 cells per 3 h, and the CV for reaction rates in triplicate wells containing control cells ranged from 4.3 to 7.3%.

Assay B (long-term incubations of intact cells). In this assay, 4.0 × 10^6 cells were incubated for 48 h with 2 ml complete medium (M-199 plus 10% calf serum) plus 90–230 pM [^{125}I]T_4 and 22–60 pM [^{131}I]T_3, or with 130–210 pM [^{125}I]rT_3, without DTT, otherwise using the same conditions described previously (assay A). After the incubation, media and cell extracts were processed as in assay A. [^{125}I]T_3 was isolated by descending paper chromatography in TAA. The lower limit of sensitivity in these conditions was 0.5% conversion per 4 × 10^6 cells/48 h. The CVs for the blank wells and reaction rates in control cells ranged from 1.4 to 11% and 5.1 to 7.7%, respectively. The differences between assays A and B were the presence of calf serum, the absence of DTT, and the longer incubation time in assay B.

The possibility of retention of newly produced [^{125}I]T_3 in cells above amounts predicted from the distribution of [^{131}I]T_3 added to the medium was assessed in some experiments using assays A and B, by calculating an R ratio (17), equal to $\{[(^{125}I)T_{3\ cells} \div (^{125}I)T_{3\ medium}] \div [(^{131}I)T_{3\ cells} \div (^{131}I)T_{3\ medium}]\}$.

Assay C (column assay of sonicates and homogenates). Cells preincubated under different conditions were scraped off the dishes, centrifuged at 1,000 rpm, and sonicated using a 225R 200-W sonicator with microtip (Heat Systems Ultrasonics, Farmingdale, NY) at medium setting in 500 μl of 10 mM Hepes, pH 7.0, 0.32 M sucrose, 10 mM DTT (HSD buffer). The cell sonicates were frozen and kept at −20°C until they were assayed, always within 10 d. In some experiments, minces of the starting membranes were homogenized in 10 vol of HSD buffer and assayed similarly.

5'-deiodinase was determined by release of $^{125}I^-$ from [^{125}I]T_4, quantitated by ion exchange chromatography on Dowex 50W-X2 (Bio-Rad Laboratories, Richmond, CA) (18). Samples were assayed in duplicate at 37°C for 2 h, after mixing 50 μl of cell sonicate containing 25–75 μg protein (in HSD buffer) with 50 μl of substrate solution. The final incubation mixture contained 20 mM DTT, 1 mM EDTA, 1 nM T_4, and 1 μM nonradioactive T_3 (to prevent deiodination of newly formed [^{125}I]T_3) (19). Preliminary experiments using paper chromatographic analysis established that under these conditions, equal amounts of ^{125}I- and [^{125}I]T_3 were produced from [^{125}I]T_4. Previous studies showed that product formation was linear with time for 2 h, and that at the proteins concentrations used, the reaction was proportional to protein concentration (1). Buffer blanks and inactivated cells blanks (immersed in boiling water for 30 min before assay) gave identical results after 2-h incubations, and one or both were included in each assay. Total 5'-deiodinase activity was the result of incubations carried out without any inhibitor. The lower limit of sensitivity of assay C was 10 fmol/h per mg protein. CVs for blanks and rates in sonicates of control cells ranged from 2.4 to 12.2% and 1.6 to 12.0%, respectively.

Thyroid hormone-depleted calf serum (THDCS). Calf serum was depleted of thyroid hormones after the protocol of Samuels (20) using the ion exchange resin IRA-400 (Mallinckrodt Inc., St. Louis, MO). The efficiency of the procedure, assessed by the addition of a small amount of [^{125}I]T_3 to the calf serum, was 90–94%. After the resin was removed, the serum was dialyzed overnight against 40 vol of 0.01 M $NaPO_4$ (pH 7.4) with 0.9% NaCl. By radioimmunoassay (RIA), calf serum T_4 and T_3 concentrations were 8.1±0.2 μg/dl and 170±5 ng/dl, respectively, before resin treatment. After the resin treatment, T_4 and T_3 values fell to 2.0±0.3 μg/dl and <30 ng/dl.

Other assays. Prolactin (PRL) was measured by a specific homologous RIA. Human PRL antiserum was provided by Dr. A. F. Parlow (Harbor-UCLA Medical Center, Torrance, CA), and [^{125}I]PRL with 50 μCi/μg of specific activity was obtained from Cambridge Medical Diagnostics, Inc. (Billerica, MA). The lower limit of sensitivity of the assay was 1.0 ng/ml, and the interassay CV was 3.0% for a serum with a mean value of 25.5 ng/ml. The cross-reactivity of the anti-PRL antibody with beta subunit of human chorionic gonadotropin (βhCG) and human placental lactogen was below 0.01%. PRL levels in fresh (not incubated with cells) M-199 with either 10% calf serum or 10% THDCS were undetectable.

βhCG was measured by a specific homologous RIA using a kit from Corning Medical and Scientific (Medfield, MA). The lower limit of sensitivity of the assay was 3.0 mIU/ml. Fresh M-199 (not previously incubated with cells) supplemented either with 10% calf serum or 10% THDCS had undetectable levels of βhCG.

Protein was measured by the method of Bradford (21) using reagents from Bio-Rad Laboratories, and bovine γ-globulin as standard. DNA was measured by the method of Burton (22) as modified by Giles and Myers (23). Results are expressed as mean±SD. Statistical analysis included analysis of variance, followed by Tukey's honestly significant difference test (24), and unpaired *t* statistics.

Results

Cell dispersion conditions. T_4-5′-deiodination measured by assay C, in cells liberated after the digestion, was expressed relative to the rate per milligram protein in sonicates of the same tissue before digestion. Cells liberated during the first 30 min of digestion had very low activity and were discarded. The number of viable cells liberated during the digestion increased with concentrations of collagenase up to 90 U/ml, whereas concentrations of trypsin higher or lower than 0.1% resulted in a smaller cell yield. Similarly, 5′-deiodination rates were highest using 0.1% trypsin and 67.5 U collagenase/ml. The T_4-5′-deiodination in cells liberated by all collagenases was similar, ranging from 1.8 to 2.5 times the activity of homogenates of the same tissue before dispersion. However, the number of cells released by the different collagenases varied widely, ranging from 0.3 to 4.8 × 10^6 cells/g membrane wet weight. Collagenases Sigma type V and VII, and Worthington type II had the greatest yield of cells. DNAse I was added at 8 μg/ml to the enzyme mixture in the last 10 min of incubation to avoid the development of cell clusters. This addition did not alter the specific activity of 5′-deiodination in the cell sonicates. In light of these results, in all experiments described below, a solution with 0.1% trypsin, 67.5 U/ml of collagenase (Sigma type VII or Worthington type II), and 8 μg/ml of DNAse I was used. The product of this digestion was composed of two morphologically distinct types of cells. The predominant type (~90%) was round and the other was fusiform; no further attempt was made to identify the different cell types.

Characteristics of the 5′-deiodination reaction in the different deiodinase assays. In assay A, when freshly dispersed cells were incubated for 3 h with [^{125}I]T_4 and [^{131}I]T_3 in the presence of 10 mM DTT, the fractional conversion of T_4 to T_3 amounted to 4–16%/10^6 cells in six dispersions, with a mean of 12.6%. Consumption of T_4 averaged 19% with a range of 14–22%. Degradation of added [^{131}I]T_3 in the same incubations was 0–6%. T_4 5′-deiodination was linear with time up to 3 h. The R ratio was always near 1.0, indicating that there was no selective accumulation of the newly formed [^{125}I]T_3 inside the cells. Fig. 1 shows the effects of altered incubation conditions on the T_4 to T_3 conversion rate in assay A. Reductions to half the number of cells caused a decrease to 60% of control in the conversion rate, $P < 0.05$. Cells immersed in boiling water for 30 min had rates < 5% of that of controls, $P < 0.001$. When 0.1% bovine serum albumin was added to the system, the rate was reduced to 45% of the control rate, $P < 0.005$, presumably due to a reduction of the free fraction of the substrate. Addition of 50 nM iopanoic acid to the incubation medium reduced the deiodination to 40% of the control level, $P < 0.005$, and 1 mM 6-*n*-propylthiouracil (PTU) decreased the conversion rate by 12–15% in three experiments, P not significant.

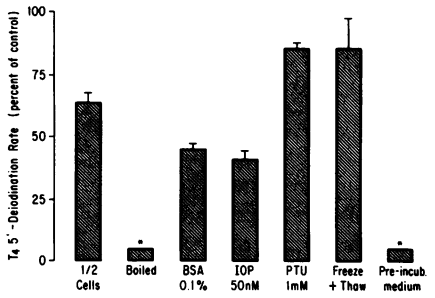

Figure 1. Effect of altered incubations on T_4 5′-deiodinase activity. Deiodination was measured by assay A in triplicate wells containing 2 × 10^6 cells, 2 ml medium, and 120 pM [^{125}I]T_4, using 3-h incubations. Values are percent±SD of the mean value in simultaneous triplicate wells not subjected to any manipulation. Bar labels: ½ cells, 10^6 cells/well; boiled, cells immersed in boiling water for 30 min before assay; BSA, bovine serum albumin; IOP, iopanoic acid; freeze + thaw, cells frozen at −60°C and thawed three times before assay; pre-incub. medium, cell-free medium preincubated for 3 h with 2 × 10^6 cells. *T_4 5′-deiodination rates were less than the lower limit of sensitivity of the assay, which was 5% of the rate in control wells in these experiments.

In two experiments, 48 h after cells were plated, medium was removed and the petri dishes were allowed to dry at 37°C. The cells were then frozen at −60°C, then thawed. The freezing and thawing was repeated another two times, after which deiodinase activity was measured by assay A. No change in T_4 5′-deiodination was seen when results were compared with control cells (Fig. 1).

In order to verify that deiodination was occurring in the cells, i.e., that the enzyme was not released into the medium, the following experiment was carried out. 48 h after the cells were plated, medium was changed and cells were preincubated with serum-free M-199 (without DTT or tracer) for 3 h, after which the preincubation medium was removed and saved, and fresh serum-free medium was added. Then 200 μl of M-199 containing DTT and [^{125}I]T_4 was added to dishes containing (*a*) cells with fresh medium; (*b*) cell-free medium previously incubated for 3 h with cells; or (*c*) cell-free fresh medium. Preincubation medium and fresh medium both had undetectable 5′-deiodination rates (Fig. 1).

The effects of addition of thyroid hormones on the deiodination rate in assay A are shown in Table I. 10- and 50-nm concentrations of nonradioactive T_4 caused a progressive fall in fractional 5′-deiodination, with 50% inhibition occurring below 10 nM, although the total T_3 production (fractional conversion × [T_4]) increased with higher substrate concentrations. No reduction in the fractional conversion was seen in the presence of 100 nM T_3.

The effect of increasing DTT concentrations on the deiodination rate in assay A is shown in Table II. Cells incubated

Table I. Effect of Thyroid Hormones on T_4 5′-Deiodination in Assay A

	[T_4]	[T_3]	Fractional conversion	Total T_3 production
	nM	*nM*	*% Conversion/10^6 cells/3 h*	*fmol/10^6 cells/3 h*
Exp I	Tracer	Tracer	8.6±0.6	40±2
	+10	Tracer	3.4±0.3*	680±60
	+50	Tracer	1.2±0.3*‡	1,200±60
Exp II	Tracer	Tracer	14.3±1.0	66±4
	Tracer	+100	16.5±1.0	76±4

Values represent mean±SD of triplicate wells. Deiodination was measured by assay A. Two million freshly dispersed cells were incubated in a 2-ml vol with 230 pM [^{125}I]T_4 and 23 pM [^{131}I]T_3, and variable concentrations of nonradioactive T_4 or T_3.
* $P < 0.001$ vs. tracer alone by Tukey's test.
‡ $P < 0.001$ vs. 10 nM T_4.

Table II. Effects of DTT and PTU on T_4 5'-Deiodinase Activity

Concentration	T_4 5'-Deiodination rate
mM	
DTT 1	2.6±0.2*
DTT 4	6.4±0.3*
DTT 10	12.7±1.6*
DTT 20	17.6±0.7*
PTU 0	100.0±4.4‡
PTU 0.01	97.0±0.6‡
PTU 0.1	94.0±1.6‡
PTU 1.0	89.6±0.4‡
PTU 5.0	70.1±3.0‡

Values are expressed as mean±SD.
* Deiodination, expressed as percentage conversion per 10^6 cells/3 h, was measured by assay A in triplicate wells containing 2×10^6 cells, 2 ml medium, and 73 pM [^{125}I]T_4.
‡ A pool of cell sonicates, derived from three different placentas, was prepared. Deiodinase activity was measured in triplicate by assay C. PTU was added only at the time of the assay. Values are expressed as percentage of the mean value obtained in assays done without any inhibitor.

Table III. Effect of Length of Culture on T_4 5'-Deiodination Rate

Sample	Time (h after plating)	T_4 5'-Deiodination rate
		fmol/h/mg protein
Membrane homogenate	0	48±5
Cell sonicates	0	76±5
Cell sonicates	24	29±1*
Cell sonicates	48	74±3
Cell sonicates	72	80±7
Cell sonicates	96	91±9
Cell sonicates	120	77±3
Cell sonicates	144	96±1
Cell sonicates	168	50±3
Cell sonicates	192	38±1*

Results are mean±SD of triplicate homogenates or sonicates from triplicate wells, using assay C. Medium was changed every 48 h. Values marked with * differ from all others except 168 h and each other at $P < 0.05$ by Tukey's test.

with 4 mM DTT generated 50% of the amount of T_3 produced by cells in 10 mM DTT. Although 20 mM DTT increased the deiodination rate above the rate at 10 mM DTT, it caused also a major cell loss: at the end of a 3-h incubation, there were 40% fewer cells in the wells incubated with 20 mM DTT than in those incubated with 10 mM, whereas little or no difference in cell number was seen comparing 10 mM DTT and lower concentrations.

In assay B, in three experiments carried out in the presence of 10% calf serum and without DTT, 4.0×10^6 cells incubated for 48 h converted 6.1±0.7% of the T_4 into T_3. During the same incubations, 15.6% of the [^{125}I]T_4 and 23.2% of the [^{131}I]T_3 were consumed. In three experiments, addition of 1 mM PTU to the wells reduced the T_4 to T_3 conversion rate by only 9.6±7.6%, P not significant. Using a similar protocol, but with [^{125}I]rT_3 instead of [^{125}I]T_4, there was 8.0±0.6% (n = 2) conversion of rT_3 into 3,3'-L-diiodothyronine by 4×10^6 cells in 48 h.

In assay C, when cell sonicates were assayed in the presence of 1 nM T_4, 5'-deiodination rates ranged from 61 to 132 fmol/h per mg protein. In 10 incubations, inclusion of 1 nM T_4 decreased the fractional conversion to 87±8% of the rate in incubations carried out with tracer only (~0.15 nM [^{125}I]T_4). Concentrations of PTU from 10 μM to 1 mM added to assay C incubations had no significant inhibitory effect, but 5 mM PTU caused a 30% inhibition of T_4 5'-deiodination (Table II), $P < 0.01$ by Tukey's test.

Studies using cells in primary culture. 24 h after the cells were plated, there was marked reduction in the deiodination rate, then a recovery of activity which persisted through the sixth day, and a subsequent decline (Table III). The production of T_3 (assay A) increased approximately in proportion to the cell density up to ~3.0×10^6 cells/25 cm^2 dish, then levelled off. The following experiments were carried out between 48 and 144 h after the cells were plated at an initial cell density of 3.0–4.0 $\times 10^6$ cells/25 cm^2 dish. Although no attempt was made to assess cell multiplication, 192–240 h after 4.0×10^6 cells were plated in a 25-cm^2 dish the cells reached confluence, whereas 24 h after plating, by visual estimate, only half of the dish surface was occupied.

Effects of thyroid hormone deprivation. After incubation of dispersed placental cells for 2 d in complete medium with normal calf serum, the medium was changed, and cells were incubated with medium containing either 10% THDCS or 10% normal calf serum. After another 48 h, deiodination was measured by assay A (Table IV). The cells that had been kept previously in medium with THDCS had a 64.2±11.6% increase in the deiodination rate above those incubated with normal calf serum. Similarly, in assay B, the thyroid hormone-depleted cells showed a twofold increase in the T_4 to T_3 conversion rate when tracer T_4 and T_3 were added to the medium at the beginning of the second 48-h incubation (Table IV). When assay B incubations were done with tracer rT_3, the cells kept in a medium with THDCS had an 85.0±5.0% increase above those kept in normal calf serum (data not shown).

Lastly, cells were collected and washed, after a 48-h incubation, either with normal calf serum or THDCS, and cell sonicates were assayed in the presence of 1 nM T_4 (assay C). The T_4 5'-deiodination rate in sonicates of cells incubated in thyroid hormone-depleted medium was 81±14% greater than that in cells incubated with normal serum (Table IV). The added T_4 concentration in assay C, 1 nM, was much greater than that contributed by the T_4 content of cells cultured in either type of serum: 125±25 pM from cells in normal calf serum and 26±6 pM from cells in THDCS, as monitored by tracer T_4 added to parallel wells.[2]

Thus, all three assays showed a near doubling of 5'-deiodination after 48 h deprivation of thyroid hormone. The increase

2. These concentrations were calculated using wells incubated in parallel to the experimental wells, with the addition of tracer T_4. At the end of the culture incubations, cells from these wells were collected, washed, and pelletted as for the experimental cells. The pellets were counted, extracted with ethanol, and chromatographed to verify that the radioactivity was still almost all T_4. From the percentage of total [^{125}I]T_4 counts found in the pellet and the known amount of nonradioactive T_4 in each well, the gravimetric content of T_4 in the pellet was calculated, and from that the concentration of T_4 in sonicate was calculated.

Table IV. Effect of Thyroid Hormone Deprivation on T_4 5'-Deiodination

Assay	n	Deiodination rate	
		Control medium	T_3/T_4 Depleted medium
Rate in assay A, %/4 × 10^6 cells per 3 h (3 h, intact cells, 10 mM DTT)	3	19.0±1.4	31.2±2.2
Rate in assay B, %/4 × 10^6 cells per 48 h (48 h, intact cells, no DTT)	3	6.1±0.7	12.2±0.8
Rate in assay C, fmol/h per mg protein (2 h, cell sonicate)	4	127±22	230±18

Cells were incubated for 48 h in medium containing 10% calf serum or 10% THDCS. In assays A and C, deiodination was measured after the 48 h, while in assay B deiodinase activity was measured during the 48 h. Results are mean±SD of individual experimental means. Within each experiment, the difference in deiodination rates between cells incubated in the two media was significant, with $P < 0.002$ by the t test.

was not due to a difference in tracer T_4 specific activity, as judged by assay C results, and did not require the presence of DTT, as judged by assay B results. In both assay A and C, when results were expressed per microgram DNA instead of per milligram protein, the magnitude of the differences in activity induced by calf serum and THDCS were similar to those in Table IV (data not shown).

In time-course studies (Fig. 2), placental cells incubated in a thyroid hormone-depleted medium had significantly higher activity than the controls as early as 12 h after the media were changed, and at all later times. There was little further change in the activity between 36 and 96 h.

To assess whether the rise in 5'-deiodination in the cells incubated with resin-treated serum was due only to thyroid hormone deprivation, two experiments were carried out. In the first, cells were kept in M-199 with 10% calf serum for 48 h, then incubated with medium containing 10% calf serum, 10% THDCS, or 10% THDCS supplemented with different concentrations of T_4, T_3, or rT_3 for an additional 48 h. Cells were then collected, washed, sonicated, and assayed for T_4 5'-deiodination by assay C. Increasing concentrations of all three iodothyronines caused a progressive impairment in the rise of 5'-deiodination induced by the thyroid hormone deprivation (Fig. 3). On a molar basis, T_3 was 2–3 times less potent than T_4, whereas T_4 and rT_3 were approximately equipotent.

In the second experiment, the medium was changed at 48 h to medium containing either 10% calf serum or 10% THDCS. After another 48 h, a second medium change was carried out. The cells incubated in 10% calf serum were kept in the same medium, whereas those kept in 10% THDCS were divided into four groups, in which the M-199 was supplemented with 10% THDCS, 10% THDCS plus 13 nM T_4, 10% THDCS plus 120 nM T_3, or 10% THDCS plus 60 nM rT_3. The incubations were stopped at timed intervals thereafter, and deiodination was

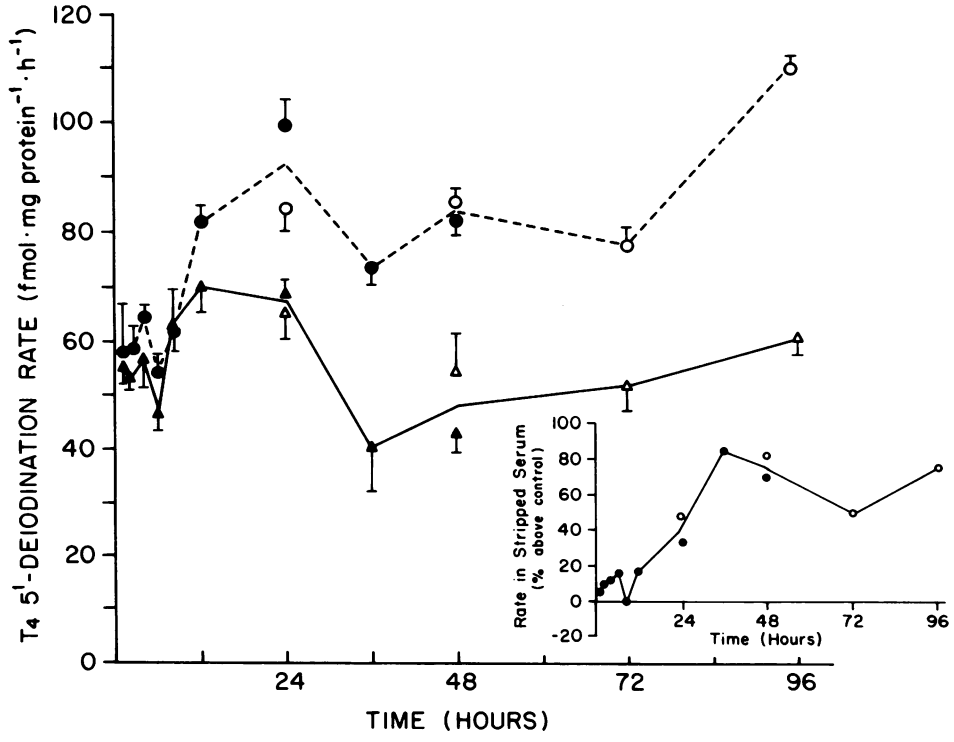

Figure 2. Time course of rise of T_4 5'-deiodination rate induced by thyroid hormone deprivation. In two experiments, cells were incubated in M-199 supplemented with either 10% calf serum (triangles) or 10% THDCS (circles). In the first experiment (closed symbols), the longest incubation was 48 h, while in the second (open symbols) the longest was 96 h. Deiodination was measured by assay C and data are expressed as mean±SD of triplicate wells, each assayed in duplicate. (*Inset*) The same deiodination rates in cells incubated in 10% THDCS, expressed as percentage above the mean value in cells simultaneously harvested from 10% calf serum.

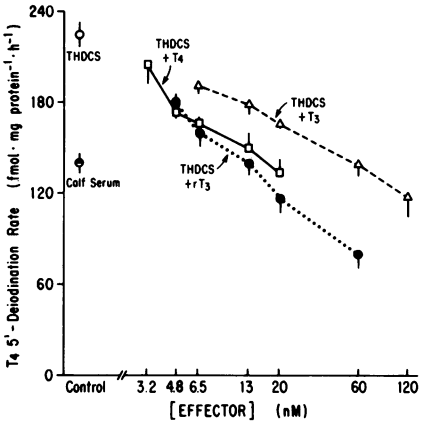

Figure 3. Inhibition by T_4, T_3, and rT_3 of the rise in T_4 5'-deiodination. Cells were incubated for 48 h with M-199 containing 10% calf serum (●), 10% THDCS (○), or 10% THDCS supplemented with T_4 (□), T_3 (△), or rT_3 (●). Deiodination was measured by assay C and data are expressed as mean±SD of triplicate wells, each assayed in duplicate. The scale of the abcissa is logarithmic.

measured by assay C. Addition of each of the iodothyronines caused a time dependent, progressive fall of 5'-deiodination (Fig. 4). 8 h after the last medium change, cells incubated with THDCS supplemented with T_4, T_3, or rT_3 showed deiodination rates similar to that of cells kept in normal calf serum (~78% of the activity of cells in THDCS only). Subsequently, cells kept in a THDCS medium supplemented with the different iodothyronines had deiodination rates lower than those of cells kept in medium either with THDCS or normal calf serum. At the end of 24 h incubation, 5'-deiodination in cells kept in THDCS supplemented with T_4, T_3, or rT_3 was respectively 60.3±6.4%, 49.9±3.0%, and 55.0±5.8% of the values seen in cells incubated

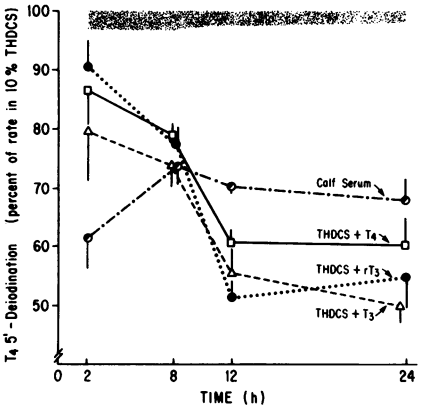

Figure 4. Time course of the fall in T_4 5'-deiodination after thyroid hormone repletion. Cells were preincubated in M-199 supplemented either with 10% calf serum or 10% THDCS for 48 h. Medium was then changed. Cells in 10% calf serum were kept in the same medium (○), whereas those in 10% THDCS were supplemented with 10% THDCS, 10% THDCS + 13 nM T_4 (□), 10% THDCS + 120 nM T_3 (△), or 10% THDCS + 60 nM rT_3 (●). Incubations were stopped at the indicated times, and deiodination was measured in triplicate wells by assay C. Data are expressed as percent±SD of the mean value in cells incubated with unsupplemented THDCS. The shaded area indicates mean − 1 SD for the latter cells, which were also harvested at the indicated times.

with THDCS only. Although the time course of fall of 5'-deiodination induced by all iodothyronines was similar, the fall did not appear to follow a simple exponential decay, and the data were not sufficiently precise for multi-component curve fitting.

Effects of thyroid hormone depletion on medium prolactin and chorionic gonadotropin. Dispersed human chorion-decidual cells produced PRL and βhCG and released them into the medium. The secretion of PRL was reasonably constant throughout the 96-h incubation period, whereas secretion of βhCG was greatly diminished in the second 48-h period (Table V). There was little difference between PRL release in wells containing medium with 10% calf serum and medium with 10% THDCS (Table V). When results of this experiment and a similar one (not shown) were combined, and data from all time points pooled, PRL secretion by cells in hormone-depleted medium was 12% greater than in control wells. Likewise, in two experiments, one shown in Table V, βhCG secretion was similar in wells containing control medium and thyroid hormone depletion medium.

Discussion

In the present studies we determined the optimal dispersion conditions to obtain viable, dispersed, human, chorion-decidual cells that retain T_4 5'-deiodinase activity. The characteristics of deiodination by these intact cells are similar to those described for human chorion-decidual membrane homogenates and microsomes (1). 50% inhibition of fractional T_4 deiodination occurs at a T_4 concentration <10 nM, and 1 mM PTU has little effect on the deiodination rate. Response of this placental T_4 5'-deiodination reaction to short term (3 h) exposure to DTT, PTU, T_3, and iopanoic acid are also very similar to those seen in homogenates or microsomes of rat anterior pituitary, brain, brown adipose tissue, and placenta (1, 3–6). T_4 5'-deiodination in these latter tissues has been termed type II iodothyronine deiodination to distinguish this pathway from another, type I, which is found most abundantly in rat liver and kidney (8).

The lack of reduction of T_4 5'-deiodination in cells submitted to the freezing-thawing process indicates that, at least at supraphysiologic thiol concentrations, integrity of cell architecture does not potentiate deiodinase activity, although in the intact cell system there is no leak of active enzyme into the culture medium. The relatively short persistence, 6 d, of 5'-deiodination activity in human chorion-decidual cells kept in primary culture, could possibly be due to overgrowth of cells lacking the capacity for 5'-deiodination. The placental membrane used as the source of the cells is composed of several different cell types, which have two different origins: cells in the chorionic membrane proper, of which there are multiple varieties (25), are of fetal origin, whereas the cells in the adherent decidual layer are of maternal origin. It is not known which of these cell types carry out 5'-deiodination.

Three different assays were used to measure T_4 5'-deiodination, because each has strong and weak aspects. Assay C (column assay of cell sonicates) is technically easiest, but has the disadvantage of using a broken cell system. Assays A (short-term incubation of intact cells) and B (48 h incubation of intact cells) are similar; assay B does not require the addition of the unphysiologic DTT, but is longer and results in lower deiodination rates. Of note is the minimal inhibition of T_4 5'-deiodination by 1 mM PTU in all three assays, particularly in assay

Table V. PRL and βhCG Levels Release by Chorion-Decidual Cells in Primary Culture

	PRL		βhCG	
Time	Control medium	T_3/T_4 Depleted medium	Control medium	T_3/T_4 Depleted medium
h	*ng/mg cell protein*	*ng/mg cell protein*	*mIU/mg cell protein*	*mIU/mg cell protein*
24	14.0±0.7	20.1±2.2	19.6±3.2	22.9±5.4
48	45.1±3.8	51.5±3.2	30.5±7.8	33.7±2.7
72	16.5±0.9	15.6±0.8	—*	—*
96	30.8±3.5	40.1±2.5	12.2±0.9	<10.0

Values are mean±SD of triplicate wells. During attachment, cells were incubated in medium with 10% calf serum. Thereafter, half the cells received medium with 10% THDCS, and medium was changed in all wells every 48 h. Thus, the 48- and 96-h values represent hormonal secretion during the first and second 48-h periods after attachment, while the 24- and 72-h values represent the first half of those periods. The amount of hormone released into the medium was corrected for the total cellular protein of each well. The lower limits of detectability for typical wells were 3.7–4.0 ng PRL/mg cell protein and 10–12 mIU βhCG/mg cell protein. * Not measured.

B, in which there was no DTT to potentially block a PTU effect. This strengthens the argument that type II deiodination has a vastly lower sensitivity to PTU than the type I reaction, independent of assay conditions.

A major finding in these experiments is regulation by thyroid hormones of human placental type II T_4 5′-deiodination. Thyroid hormone deprivation clearly induced a rise in 5′-deiodination, while repletion caused a fall in the activity. There are two different mechanisms of reduction of fractional T_4 deiodination by addition of iodothyronines. In assay A, addition of T_4 greatly reduces the tracer specific activity, and, despite low fractional T_4 deiodination, the molar rate of T_3 production increases with increasing T_4 concentrations (cf. Table I). This merely represents saturation of the enzyme by substrate. In this system, addition of 100 nM T_3 had no effect.

In contrast, when cells previously kept in 10% THDCS were incubated in the same medium supplemented with T_4, T_3, or rT_3, the consequent marked reduction in T_4 5′-deiodination truly reflected decreased enzyme activity, because tracer specific activity was not altered substantially during the deiodinase assay (assay C). Here, additions of T_3, as well as of T_4 and rT_3, to THDCS did have effects, which were namely to prevent the rise in deiodination rate induced by thyroid hormone deprivation and to reverse an increased activity already established. That these effects are really hormonal, or biological in nature, not merely a reflection of enzyme kinetics, is further suggested by their occurrence at physiological T_4 concentrations. Although we did not directly measure the free T_4 and free T_3 concentrations in culture media, dilution of the calf serum in M-199 should keep the free T_4 and free T_3 concentrations very nearly the same as in whole serum. Thus, the total T_4 concentration in M-199 with 10% calf serum, 10.4 nM, corresponds to a physiological free T_4 concentration, and restoration of similar amounts of T_4 to the hormone-depleted serum abolishes its capacity to induce and maintain raised T_4 5′-deiodinase activity. For T_3, in contrast, prevention of the rise in enzyme activity requires a 60-nM concentration, >200 times the level in medium containing 10% normal calf serum, 0.26 nM T_3.

Leonard and Larsen (26), studying type II T_4 5′-deiodination in cultured fetal rat brain cells, found that reaction rates in cells cultured in medium containing iodothyronine-stripped serum was about twofold higher than in cells cultured in the presence of normal serum. Addition of 100 nM T_4 or T_3 prevented the increase in activity. Similarly, they found that PTU-insensitive rT_3 5′-deiodination was increased in the presence of stripped serum, and this increase was partly prevented by 10 or 100 nM T_3. They did not test lower concentrations. In our system, T_4 and T_3 appear to be more potent, in that lower concentrations reversed the effects of stripped serum.

The relative potencies of T_4, T_3, and rT_3, and the small amount of T_4 degradation in 48 h of culture (assay B), indicate that the effect of T_4 could not be explained by its transformation to T_3 or rT_3. Although T_3 is a more potent thyroid hormone than T_4 in most respects, and although rT_3 is not calorigenically active, there are some other systems in which T_4 is as potent as T_3 (27, 28), and some in which rT_3 has effects similar to T_4 and T_3 (27, 29). The relative potencies we find for these three iodothyronines are in notably good agreement with the results of Silva and Leonard (30) regarding changes in rat brain and pituitary type II deiodinase after in vivo administration of T_4, T_3, and rT_3.

The mechanism of increased T_4 5′-deiodination induced in these cells by thyroid hormone deprivation is not known. An increase in enzyme synthesis, decreased degradation, or activation of quiescent enzyme are among the possibilities. Leonard et al. (31) showed that hypothyroidism does not increase the synthesis of rat cerebrocortical or pituitary type II deiodinase, but rather causes a marked reduction in the enzyme turnover rate. Of considerable interest is evidence that this effect does not require protein synthesis, and therefore is presumably not initiated at the nuclear T_3 receptor (31).

These mechanisms will be amenable to testing in the human placental cell system. However, one potential artifact has been reasonably well excluded by our results: a major change in the quantitative distribution of cell types having much different deiodinase activities. First, the protein and DNA content per dish of cells incubated in each type of serum was similar (data not shown). Second, it seems improbable that an altered cell distribution promoted by thyroid hormone depletion would be corrected within 8 h by hormone repletion, whereas enzyme activity declined in this time period. Last, the amounts of prolactin, produced by decidual cells (32), and of βhCG, produced by trophoblastic cells (33), were similar for cells incubated in medium containing either type of serum. It is therefore reasonable to assume that each of these cell types comprised a similar fraction of the total cells in both normal and hormone-depleted

serum, and that it is unlikely that some cell type devoid of 5'-deiodinase activity which was a major component in medium with normal serum, became a minor component when hormone-depleted serum was used.

The rise of 5'-deiodinase activity induced by thyroid hormone deprivation appears to be slower than in the rat. Leonard et al. (31) found a twofold increase in rat cerebrocortical and pituitary type II 5'-deiodinase activity 6 h after thyroidectomy, although Maeda and Ingbar (7) found an increase in rat pituitary activity only at 24 h after thyroidectomy, not earlier. Inhibition by iodothyronines of human placental T_4 5'-deiodination previously raised by hormone depletion was also slower than in rat brain and pituitary. Rat cerebrocortical and pituitary type II 5'-deiodinase activity decreased to euthyroid levels 4 h after a single injection of a supraphysiological dose of T_3 to hypothyroid animals (31, 34).

Golander et al. (35) and Riddick et al. (36) have shown that PRL synthesized and secreted by human decidual tissue is indistinguishable from pituitary PRL by chemical, immunological, and receptor binding criteria, but unlike pituitary PRL, decidual PRL secretion is not under dopaminergic or thyrotropin-releasing hormone control (37). Our data show that decidual PRL is also unaffected by thyroid hormone deprivation, which is again unlike pituitary PRL. Release of βhCG also proved to be unaffected by 48 h of thyroid hormone deprivation.

Although the physiological role of type II 5'-deiodination in man is not established, this pathway might have an adaptive function, helping to prevent a fall in intracellular, and possible plasma, T_3 concentrations in mild-to-moderate hypothyroxinemia (8). Clinical studies offer some support for this hypothesis. Lum et al. (38) studied euthyroid subjects rendered hypothyroxinemic by exogenous T_3, and found that after withdrawal of T_3, serum T_3 levels declined only 22% below pretreatment values, while T_4 concentrations remained >50% below pretreatment levels. This phenomenon was not dependent on thyrotropin secretion, nor was it due to changes in secretory activity of the thyroid gland, as judged by serum thyrotropin measurements and similar changes in T_3/T_4 ratios in athyreotic patients after T_3 withdrawal. Fischer et al. (39) treated a group of thyrotoxic patients with either propylthiouracil or carbimazole to the point of a rise in serum thyrotropin. Serum T_3 levels fell to normal in parallel with T_4, but no further drop in T_3 was seen, despite a continued decline in the T_4 to subnormal concentrations, during the time before resumption of thyrotropin secretion was evident. The discrepancies between T_4 and T_3 in these studies suggest that there is a mechanism in man that moderates plasma, and probably intracellular, T_3 deficiency in states of diminished T_4 availability. Type II 5'-deiodination has the appropriate characteristics to play such a role, whereas type I deiodination does not (7), and the response of the low K_m kidney process (9) to hypothyroidism has not been reported.

Animal studies are also suggestive of an adaptive function for type II deiodination. In the rat cerebral cortex, cerebellum, and anterior pituitary, the majority of the intracellular T_3 is produced locally by type II 5'-deiodination of T_4, as reviewed in reference 8. Silva and Matthews (13) found that in 2-wk-old euthyroid rats, PTU-insensitive T_4 5'-deiodination was the predominant in vivo pathway to extrathyroidal T_3 production, and all such production was PTU insensitive in hypothyroid pups. Silva et al. (40) have also shown that in hypothyroid adult rats, practically all T_3 produced in vivo from low doses of exogenous T_4 was generated via a PTU-insensitive pathway.

The specific importance of type II deiodination in placental structures is not known. It is unlikely to be important for the fetus, because there is very little transfer of maternal T_4 or T_3 to the fetus (41). We speculate that the enzyme may help provide T_3 for metabolic functions in the placenta or decidua, per se. Our measurements of βhCG and prolactin were unrevealing in this regard, but little is known about thyroid hormone actions on other placental processes.

Acknowledgments

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